A CLOSED CIRCUIT APPARATUS FOR MEASUREMENT OF OXYGEN CONSUMPTION IN SMALL ANIMALS

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Summary : A simple and reliable closed circuit apparatus for short term and long term measurements of oxygen consumption of small laboratory animals is described. Repeat measurements of oxygen consumption under resting conditions of six male albino rats using this apparatus shows a variability of less than 1.5%. Use of the set-up for continuous monitoring of oxygen consumption following subcutaneous inlection of norepinephrine, for over 2.5 hr enables the measurement of energy expenditure in animals on a long term basis which is reproducible on several occasions.

Key words , oxygen consumption

closed-circuit apparatus

small animnls

INTRODUCTION

The metabolic rate of small laboratory animals may be determined by either open-or closed-circuit devices. Although many different forms of apparatus have been designed, the closed-circuit arrangement has greater advantages and convenience over the opencircuit methods which are technically some what laborious (2,8,11). Brody (5) and Swift and French (13) have reviewed the various techniques. The equipment that have been designed and described earlier are complex and expensive. The simpler ones do not permit continuous monitoring of changes in oxygen consumption over a long period of time. The main objective of this communication is to present the design and operation of a simple apparatus for monitoring oxygen consumption of small animals and give some experimental data obtained by using the apparatus.

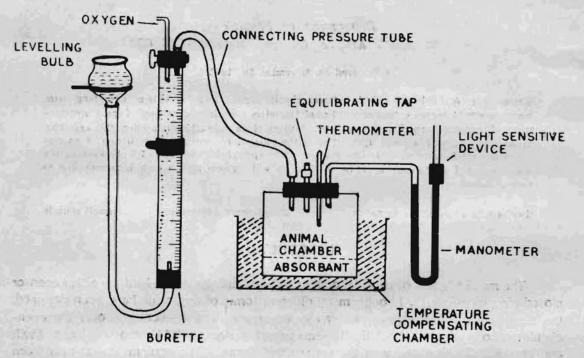
MATERIAL AND METHODS

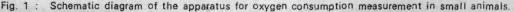
A diagram of the apparatus is shown in Fig. 1, and the construction of the apparatus is from common and readily available materials. The apparatus consists of a sealed air-tight animal chamber (ordinary rice cooker is used) containing carbon dioxide absorbent such as soda lime. This animal chamber is connected by a pressure rubber tubing to a graduated burette (25 cc) which has a three-way stop-cock. The other opening is connected to an oxygen source (a football bladder filled with oxygen will

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suffice). A water manometer is attached to the animal chamber to which a photosensitive indicator lamp is fitted which is a very useful monitor for accurate reading of the rate of oxygen consumption by the animal. A thermometer and a pressure equilibrating stop-cock is also inserted to the top of the animal chamber. The animal chamber is placed in an outer temperature compensating container so as to enable the measurement of the metabolic rate at any desired temperature.





The principle of operation of this apparatus is as follows : The pressure inside the closed chamber is raised above the ambient by delivering a predetermined volume of oxygen. Water has been utilised to push oxygen from the burette into the animal chamber (15). The rise in the chamber pressure is indicated by the change in level of the fluid in the manometer. Expired carbon dioxide and water are absorbed by the absorbent and the time taken for the chamber pressure to return to its initial level as indicated by the photosensitive lamp, gives the rate of oxygen utilization by the animal.

After checking the system for leaks, the animal is introduced into the chamber and the lid sealed. Some time (usually 10-15 minutes) is allowed for the animal to settle down and for temperature equilibration. A known volume of oxygen is delivered from the burette into the chamber. The pressure in the chamber increases and the fluid level

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in the manometer rises and the indicator lamp burns off". When the pressure drops due to oxygen consumption by the animal and reaches the initial level the lamp burns on". This interval between "off" and "on" is the time taken for oxygen consumption by the animal. The volume of oxygen consumed over a period of time is subjected to thermobarometric (STPD) corrections and the values are expressed either as volume of oxygen consumed per minute per kg body weight raised to their power 0.75 (9) or as energy expended in kilocalories (or kilojoules) per unit time per unit body weight o body surface area (1).

RESULTS

To test the validity and reproducibility of the apparatus, a short term measurement of resting oxygen consumption of six male albino rats which were fasted overnight were made for 30 min at $28\pm1^{\circ}$ C and results are shown in Table I and the results of resting oxygen consumption of rats are in agreement with values cited elsewhere in the literature (3,4,10).

Trial	Oxygen consumption		Metabolic rate
	ml/mt	ml/mt/W 0-75	Kcal/m²/hr
1	2.54±0.29	11.93 <u>+</u> 2.13	28.85 <u>+</u> 4.31
n	2.54±0.33	11.60±1.63	28.23±3.21

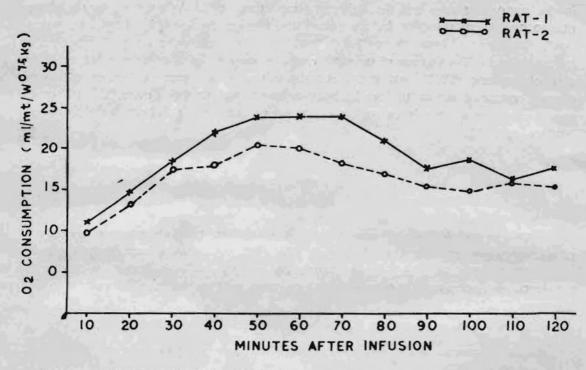
TABLE I : Comparison of measurements of oxygen consumption and metabolic rate.

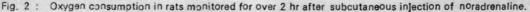
Mean +SD

n=6 (all male)

Long term measurement of oxygen consumption for over 2.5 hrs by the rats after subcutaneous injection of noradrenaline was also done. The results are shown in Fig. 2.

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DISCUSSION

A number of technniques have been described for the measurement of oxygen sonsumption of small animals (4,6,7,8,11,12,14). The apparatus used for such measurements must satisfy three basic requirements (7).

- (a) It must be comfortable enough for an animal to settle down quickly,
- (b) It must be sensitive to small changes in oxygen consumption and
- (c) Its design should permit individual and group measurements to be made on several animals under identical conditions.

A simple, economical, reliable and efficient apparatus which meets all the above requirements is described here. The various items plus accessories required are easily obtainable and the total cost incurred is less than Rs. 500/-. This is only a small fraction of the cost of other equipment for measurement of oxygen consumption of small animals. It is easy to operate and the measurements made are accurate, reliable and reproducible. Sensitivity of the apparatus is good as the dead space is minimal ; 0.5 *ml*

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of oxygen introduced into the animal chamber reflects an adequate pressure change in the manometer. Temperature equilibration is reached within 8 to 10 min of introduction of the animal and the measurements of oxygen consumption can be done under controlled temperatures by using the temperature compensating outer container.

Long term measurement of oxygen consumption is possible using this set-up and we have used the apparatus for measurements ranging from a minimum of 30 minutes to more than 180 min. This simple equipment can be constructed in any laboratory and can meet a wide range of experimental requirements. The general design of the apparatus can be adapted with suitable alterations for different laboratory animals.

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